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Effects of Exogenous Reproductive Hormones on *Haemonchus contortus* Populations in Lambs*

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ABSTRACT: The potential roles of 2 reproductive hormones, prolactin and prostaglandin, in the regulation of nematode growth, fecundity, and survival in lambs were examined in 2 experiments. In the first experiment, prolactin (25 IU/lamb) was administered to 3 groups of lambs at 1-wk intervals during each of the first 3 wk of patency of *Haemonchus contortus* infections, respectively. Fecundity (eggs/female/day) and total daily egg production were significantly higher in the group treated with prolactin during week 2 of patency. Male worms were significantly longer, and female worms were significantly shorter after each weekly treatment with prolactin. In experiment 2, a prostaglandin $F_2\alpha$ analogue, BOVILENE® (0.25 mg/lamb), was injected daily with or without exogenous prolactin (25 IU/lamb) throughout the first 3 wk of patency, the period of highest egg production. BOVILENE treatment resulted in decreased survival of both adult male and female worms and increased fecal egg concentrations, as estimated on a daily basis throughout this period. The interactions of BOVILENE with prolactin were negative relative to total daily egg production and worm growth at the terminus of the experiment (day 42 postinoculation). Additive or synergistic effects of prolactin and BOVILENE were not evident relative to fecundity. Decreased survival of worms from treatment with this potent prostaglandin analogue alone is intriguing for parasite control applications.

KEY WORDS: periparturient egg rise, prostaglandin, prolactin, parasite, nematode, growth fecundity.

An example of regulation of growth and reproduction of parasitic nematodes by the host is the phenomenon of periparturient egg rise. Increased fecal egg output is coincident with lambing and lactation in ewes with nematodiasis (Salisbury and Arundel, 1970; reviewed by Gibbs, 1986). The precise physiological cues that communicate the reproductive and immune status of the ewe have not been defined; however, exogenous prolactin, either alone or with progesterone, has been implicated as a regulatory semiochemical (i.e., a chemical that communicates between species) between lambs and their parasites. Longer term administration (2 wk) of prolactin at physiological levels throughout patency of infections of *Haemonchus contortus* resulted in increased fecundity and adult worm length (Fleming, 1993). Similarly, in ovariectomized ewes that received preinoculation injections (20 days) of progesterone followed by postinoculation injection (30 days) of prolactin, adult *H. contortus* were more numerous and larger than populations from saline-injected control lambs (Fleming and Conrad, 1989). Intravenous infusion of another hormone associated with lambing, prostaglandin $F_2\alpha$ ($PGF_2\alpha$), increased fecal egg concentrations in sheep infected with *H. contortus* (Honde and Bueno, 1983). Although these authors attributed this effect to the resumption of development of arrested fourth-stage larvae, periodic worm recoveries were not attempted to support this hypothesis.

The current experiments were designed to evaluate the temporal regulation by prolactin on growth and reproduction of *H. contortus* infections. Specifically, do discrete intervals in the parasitic adult life cycle occur when this endocrine is most effective? Additionally, the effects of a potent $PGF_2\alpha$ analogue, BOVILENE® (7-[3,5-dihydroxy-2-(3-hydroxy-4-phenoxy-1-butenyl) cyclopentyl]-4,5-heptadienoic acid) (Diamond Laboratories Inc., Des Moines, Iowa), both with and without prolactin, on growth, reproduction, and survival of *H. contortus* were examined.

Materials and Methods

Ewe lambs were raised through 60–80 days of age under worm-free conditions on concrete paddocks with hay and water provided ad libitum.

Experiment 1

Lambs (N = 30) were inoculated with 5,000 infective larvae of *H. contortus* on day 0. During the first

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3 consecutive wk of patency, beginning day 21, 28, or 35 postinoculation, groups of 10 lambs were placed in individual raised pens with suspended screens for fecal collections and daily intramuscular injections with saline (n = 5) or 25 IU (n = 5) of ovine prolactin (L-6520, Sigma Chemical Co., St. Louis, Missouri), a level that was found to increase parasite fecundity when injected over the course of the infection (Fleming and Conrad, 1989). These 3 wk of sampling correspond to the period of highest nematode fecal egg output. During each week of injections, the lambs were sampled daily for fecal egg concentrations (modified McMaster technique [Whitlock, 1948]) and total egg production, by weighing individual total fecal production. After 7 days of injections, lambs were removed from feed for 24 hr and then killed by captive-bolt gun and exsanguination. Abomasa were removed, and adult worms were rinsed with warm physiological saline and fixed immediately in 5% phosphate-buffered formalin. Adult worms were separated by sex, counted, and measured using computer-assisted digitized morphometry (Bioquant, Nashville, Tennessee). The number of female worms from this terminal collection and the average of the previous 2 days of total daily egg production per lamb were utilized to estimate fecundity (eggs/female/day). Fecal egg data were transformed to $\log_{10}+1$ and analyzed by a generalized linear model that recognized repeated sampling within an experimental unit, that is, each lamb (SAS Institute, Inc., Cary, North Carolina). Worm numbers, sizes, and fecundity were analyzed by nonparametric the Kruskal-Wallis test, and means were compared using multiple comparisons of ranked sums (Hollander and Wolfe, 1973). Differences were considered significantly different at $P < 0.05$.

Experiment 2

Three groups of lambs (n = 10/group) were inoculated with 5,000 infective *H. contortus* larvae/lamb. From days 18 to 40 postinoculation, lambs were injected intramuscularly with saline (group 1), BOVILENE (0.25 mg/lamb; group 2), or BOVILENE (0.25mg/lamb) and ovine prolactin (25 IU/lamb; Fleming, 1993; group 3). This analogue of $PGF_2\alpha$ has a biological potency 25 times that of the native compound in cattle for luteolysis. Daily fecal collections were similar to those in experiment 1. On day 40 postinoculation, lambs were removed from feed and, on day 41 postinoculation, adult worms were collected, and data were analyzed as described in experiment 1.

Results

Experiment 1

No differences occurred in nematode egg production between treatment groups throughout week 1 (days 21–28) of patency (Fig. 1). During week 2 of patency (days 28–35), prolactin treatment significantly increased total daily egg production. However, during week 3 (days 35–42), prolactin treatment significantly decreased total daily egg production. Fecundity was significantly higher at the end of week 2 of patency with prolactin administration (Fig. 2). No differences occurred in the number of males at the end of each week (data not shown); significantly fewer females occurred with treatment × time (Fig. 2). Females were significantly shorter each week after prolactin treatment; conversely, males were significantly longer with prolactin administration (Fig. 3).

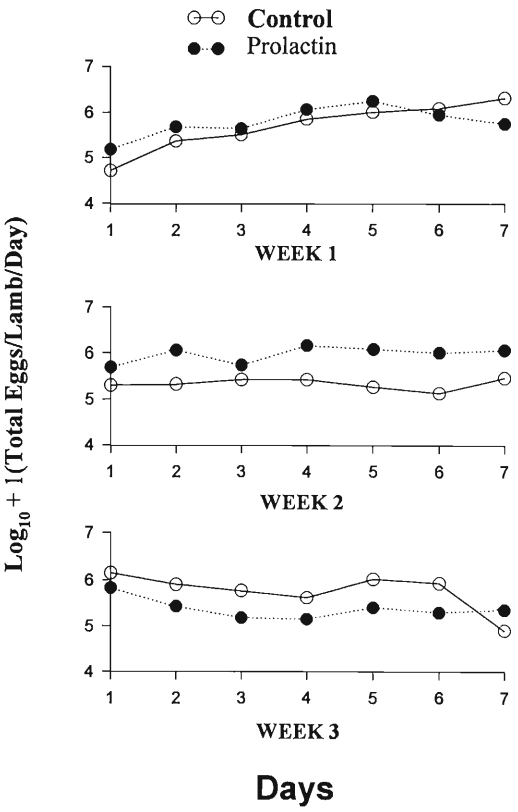

Figure 1. Logarithmic transformation of the mean total daily *H. contortus* egg production in lambs (n = 10/group) during the first 3 wk of patency with and without daily injections of ovine prolactin (25 IU/day). Lambs receiving prolactin in week 2 had significantly ($P < 0.05$) higher egg production; in week 3, egg production was significantly lower in this group (middle and lower panels, respectively).

Experiment 2

BOVILENE treatment alone significantly increased daily egg concentrations (EPG) but did not alter total daily egg production (Fig. 4, lower and upper panels, respectively). BOVILENE with prolactin resulted in shorter adult males and

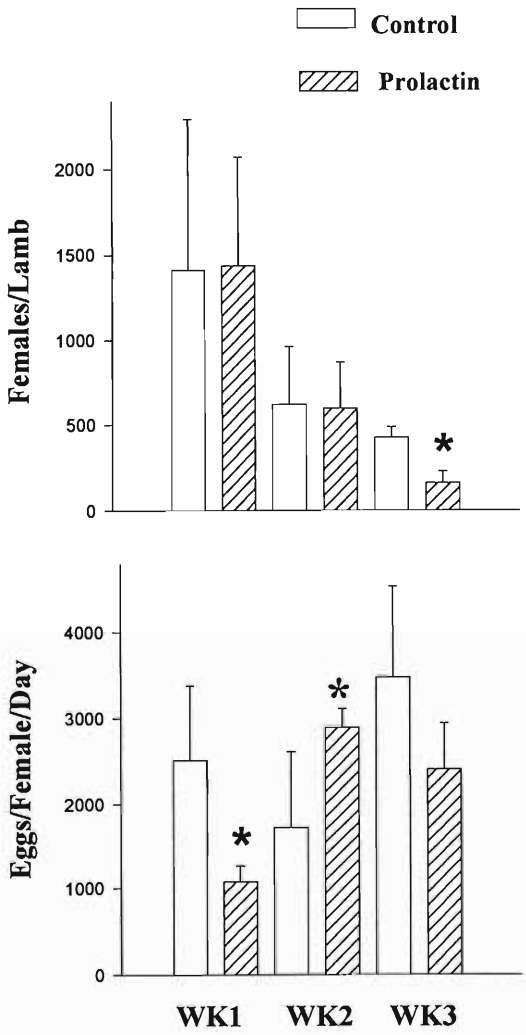

Figure 2. Mean (±SEM) number (upper panel) and fecundity (lower panel) of female *H. contortus* recovered from the abomasa of lambs (n = 10/wk) at the end of each of the first 3 wk of patency. Columns with an asterisk are significantly different (P < 0.05) than controls.

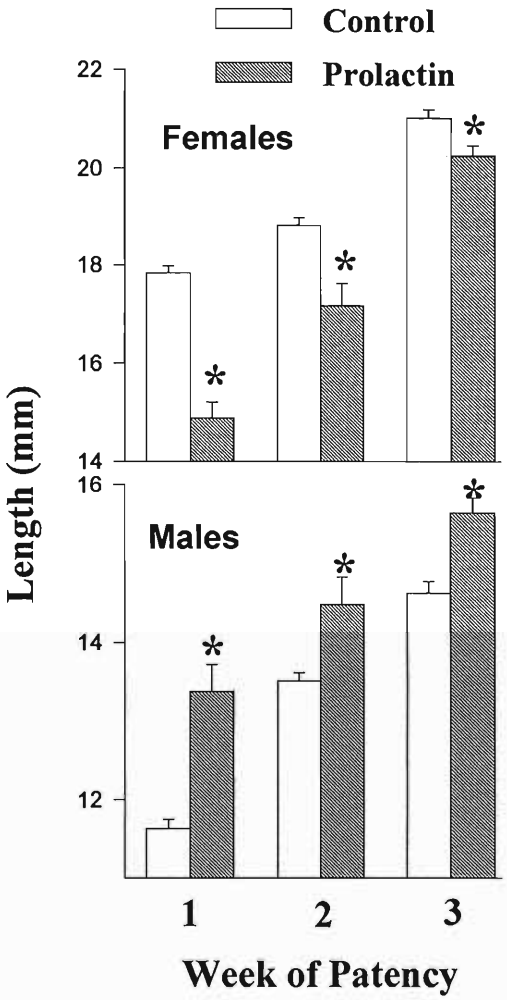

Figure 3. Length ($\bar{x}$ ± SEM; n = 25/group) of adult female (upper panel) and male (lower panel) *H. contortus* recovered from lambs (n = 10/wk) at the end of each of the first 3 wk of patency. Columns with an asterisk are significantly different (P < 0.05) than respective controls.

females (Fig. 5). No detectable differences occurred in fecundity with treatment, although BOVILENE significantly decreased survival of both male and female adult *H. contortus* (Fig. 5).

Discussion

Relatively short-term (7 day) administration of prolactin had few consistent effects on *H. contortus* populations. In contrast, fewer females and larger males occurred with more prolonged administration of prolactin (Fleming, 1993). In the current study, enhanced fecundity (eggs/female/day) with short-term treatment with prolactin was limited to the second week of patency, suggesting a shift in metabolic resources to egg production rather than growth. Similarly, enhanced fecal egg concentrations (EPG), the conventional measurement of periparturient egg rise, was identifiable only in the second week of treatment. Apparently, prolonged continuous exposure of lambs to prolactin is a requisite for consistently increased rates of egg production in

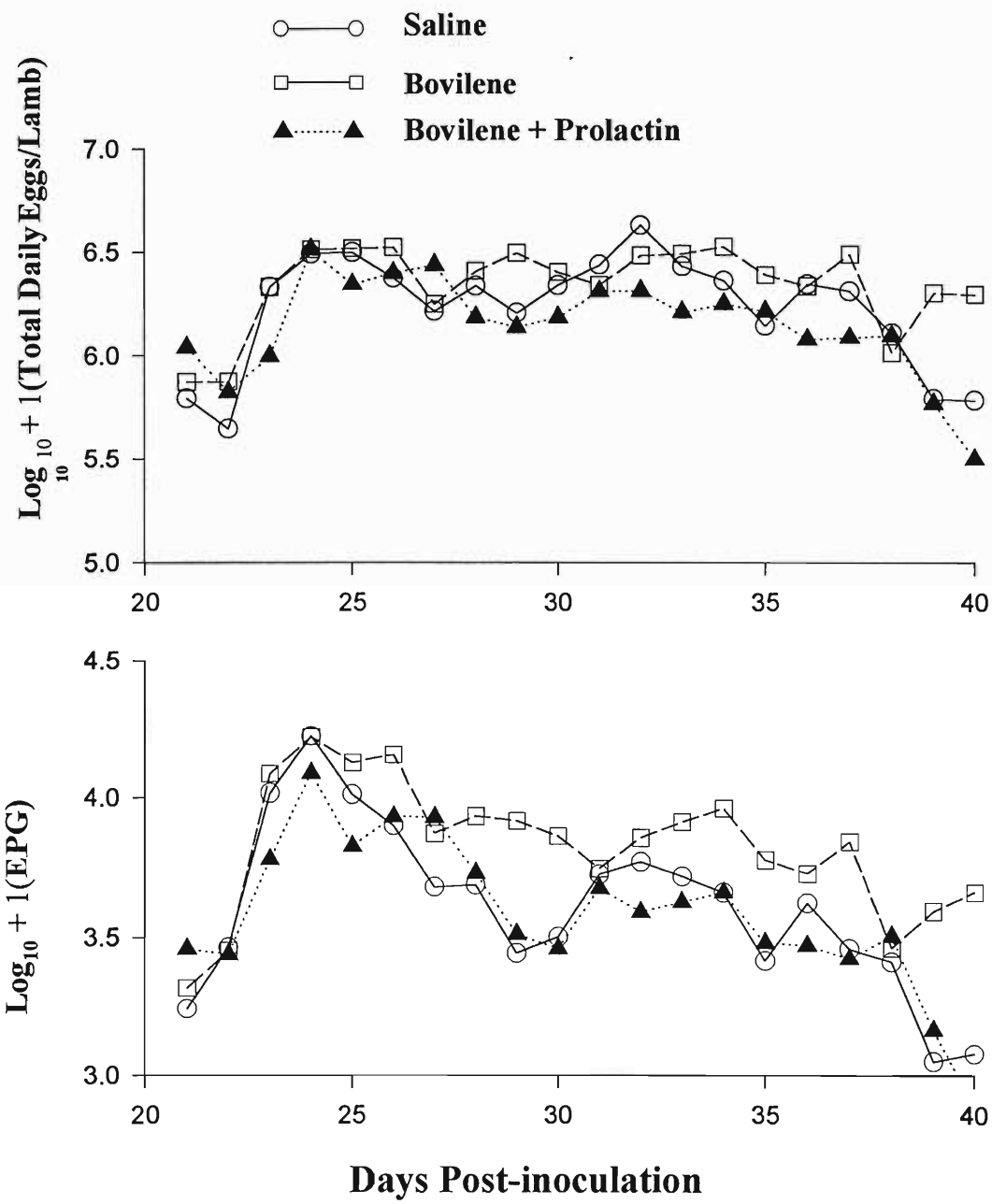

Figure 4. Logarithmic transformation of egg concentrations (lower panel) and total daily nematode egg production (upper panel) from *H. contortus* infections in lambs (n = 10/group) that were injected daily with saline, BOVILENE (0.25 mg), or BOVILENE (0.25 mg) and prolactin (25 IU) from days 20–40 postinoculation. Daily egg concentrations were significantly higher ($P < 0.05$) in the lambs receiving BOVILENE alone (lower panel).

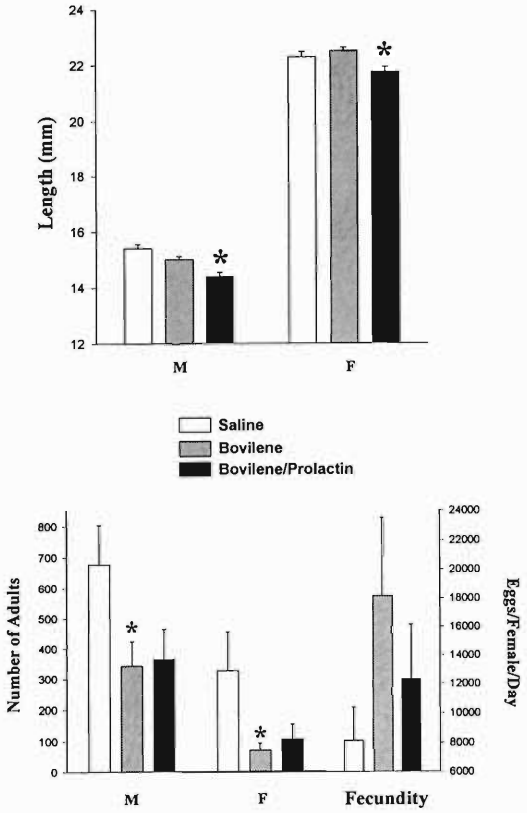

Figure 5. Lengths ($\bar{x}$ ± SEM; n = 25/group) of adults (upper panel) and adult worm numbers and fecundity (lower panel) of *H. contortus* recovered at day 41 postinoculation from lambs that were injected daily with saline, BOVILENE (0.25 mg), or BOVILENE (0.25 mg) and prolactin (25 IU) from days 20 to 40 postinoculation. Columns with an asterisk are significantly different ($P < 0.05$) than respective control groups. Abbreviations: F = females, M = male.

H. contortus infections (Fleming and Conrad, 1989; Fleming, 1993). Experimental treatment of peri- or postparturient ewes with bromocryptine (twice daily for 14 days), a potent dopamine agonist that lowers prolactin, did not alter fecal egg concentrations or the generalized immune responsiveness in sheep inoculated with *Teladorsagia* (formerly *Ostertagia*) *circumcincta* (Jeffcoate et al., 1990).

Characteristically, this gastrointestinal nematode does not have the logarithmic range in egg concentrations or fecundity that is found in *H. contortus* infections, thus perhaps limiting the opportunity to detect significant treatment differences. Similarly, the injection of exogenous steroids in nonpregnant ewes (daily for 48 days), which induced a rise in peripheral prolactin concentrations, did not induce detectable increases in egg concentrations in ewes inoculated with *T. circumcincta* (Coop et al., 1990).

Chronic daily administration of BOVILENE demonstrated increases in fecal egg concentration as demonstrated by Honde and Bueno (1983) with daily infusion of the native compound, $PGF_{2\alpha}$, although the routes of application were different (intramuscular vs. intravenous). However, the efficacy of treatment in the latter study was apparent only on day 50 postinoculation and beyond, whereas as the current study was terminated at day 41 postinoculation. Although the endogenous release of prolactin and prostaglandins are essentially simultaneous at lambing (Davis et al., 1971; Thorburn et al., 1972), no interactive effects were apparent relative to worm fecundity or growth in the current study.

BOVILENE administration was highly effective in reducing the adult worm burden by over 50%. In the bovine, $PGF_{2\alpha}$ increased contractility in the longitudinal and circular gastric muscle (Vandeplassche et al., 1982). Hence, administration of BOVILENE might enhance expulsion of the nematodes from the abomasum, thereby reducing populations of the luminal parasite. Elucidation of the mode of action of this effect, and the extent to which it occurs in other parasitic nematodes, potentially could lead to a new class of anthelminthics.

The minor alteration in most worm population parameters due to short-term prolactin and/or $PGF_{2\alpha}$ analogue treatments suggests that prolonged exposure to increased endocrine levels are requisite, at a minimum, to effect the nematode reproductive characteristics of the periparturient egg rise.

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